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14. ABSTRACT

Improvised explosive devices induce head injuries in over 40% of soldiers who survive. Increased intracranial pressure (ICP) frequently occurs in this context; successfully managing ICP improves clinical outcome, but currently requires an invasive, surgical procedure for its assay. A rapid, easy, and non-invasive method to monitor ICP would therefore be extremely useful for managing such injuries. We demonstrated in vitro that safe (as tested in vivo) ultrasound-induced palpation and, separately vibration of brain-tissue phantom correlated with the overpressure on that phantom. We demonstrated in vivo that ultrasound-induced palpation of brain varied with ICP, but not in a statistically significant fashion. In contrast, ultrasound-induced vibration of brain did vary with ICP in a statistically significant way. Improved ICP models should yield improved predictive power.

15. SUBJECT TERMS
intracranial pressure, intense focused ultrasound, vibroacoustography, acoustic radiation force, ultrasound-induced palpation

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INTRODUCTION – subject. Improvised explosive devices induce head injuries in over 40% of soldiers who survive. Increased intracranial pressure (ICP) frequently occurs in this context; successfully managing ICP improves clinical outcome, but currently requires an invasive, surgical procedure for its assay. A rapid, easy, and non-invasive method to monitor ICP would therefore be extremely useful for managing such injuries. We propose work whose ultimate goal is the replacement of the current invasive method with a non-invasive procedure that would provide a measure of ICP on or near the battlefield.

INTRODUCTION - purpose. We hypothesized that safe ultrasound-induced palpation or vibration of brain tissue, applied and monitored non-invasively through the skull, correlates with the pressure on that brain.

INTRODUCTION – scope of the research. To met the purpose of this proposal we sought to achieve three aims. **Aim #1.** We will test our methodology for inducing and monitoring acoustic brain palpation and vibration *in vitro*. **Aim #2.** We will demonstrate this methodology in a rodent model of elevated ICP. **Aim #3.** We will demonstrate the safety of this methodology using histological methods.

KEY RESEARCH ACCOMPLISHMENTS

We hypothesized that ultrasound-induced palpation of brain tissue, applied and monitored non-invasively through the skull, correlates with the pressure on that brain. We note here that 'palpation' takes on two forms. One is focal pushing of tissue under ultrasound monitoring and assay of the (a) maximum extent of the focal push or (b) the time to return to baseline of the displaced tissue. We call this procedure 'acoustic palpation.' The second consists of focally vibrating tissue with ultrasound and assaying the amplitude of the associated acoustic emissions. This second procedure is known as 'vibroacoustography.' To test our hypothesis, we have worked through the following Aims and associated milestones during Year 1 of the research program.

Aim #1. We will test our methodology for inducing and monitoring acoustic brain palpation in vitro.

Milestone #1a. We will finalize our in vitro setup for assaying our ability to induce and monitor acoustic brain palpation.

With regard to our in vitro studies of tissue palpation and vibration with intense focused ultrasound (iFU), we started by setting up our experimental tank for suspending at the focus of our iFU transducer a tissue phantom that could receive the iFU. After trying a variety of different recipes for the tissue phantom we settled on one and performed a series of studies whereby we varied the specific ultrasound frequencies, intensities and pulse durations on the phantom while applying in a simultaneous fashion dual-frequency iFU for vibroacoustography or acoustic palpation (Figure 1). For our first studies we put a calcium carbonate target in the tissue phantom: our goal was to see if the vibroacoustography method would generate higher acoustic emissions when applied to the target versus to the tissue phantom away from the target, a first test of the efficacy of our embodiment of vibroacoustography. Our results confirmed this hypothesis (Figure 2), thereby confirming our embodiment of vibroacoustography. Among the lessons learned we include the greater efficacy of using longer pulses for vibrating the target, which allowed the target to reach a state of steady vibrations, and that there can be a variety of maxima in vibration that are related to likely vibrations in the in vitro experimental setup and not the presence or absence of the target. These can create false signals, likely independent of overpressure, false signals that we will need to distinguish from signals from the tissue itself that should depend upon the overpressure on the tissue. This work on calcium carbonate targets has intrinsic merit as a proxy for kidney-stone detection as well as its primary virtue – as a means of exercising our equipment on a relatively easy problem before moving to the hard problem of relatively homogenous tissue. We are therefore pursuing publication of this work in addition to our primary focus – ICP detection.

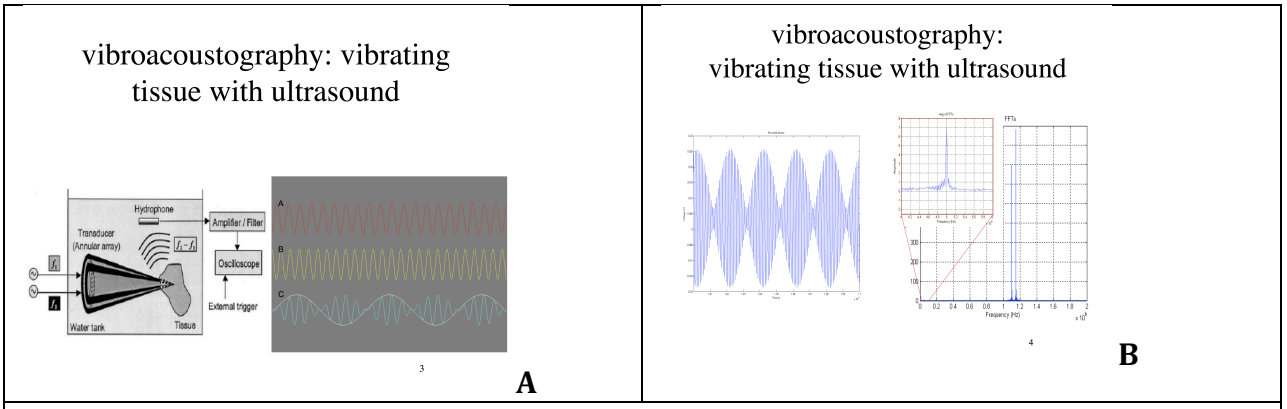


Figure 1. A. In vibroacoustography, a dual-transducer device ('transducer (annular array)') emits two, separate pulses of ultrasound at two different, but close frequencies – pulse train 'A' and 'B'. The ultrasound sources have been designed so that their foci overlap within the material of interest (tissue phantom or brain, here). At the focus, high-frequency oscillations move the tissue slightly; their beat frequency generates a low-frequency emission, itself sampled from a separate hydrophone placed either in the water tank or within the iFU source itself for *in vivo* applications. For acoustic palpation, we use the same equipment but with each transducer element running at the same carrier frequency. The result is a single, net displacement of tissue or tissue phantom, as shown below. **B.** This figure shows real data rather than the conceptualization of Figure 1A. Specifically, the left-hand figure of B shows a measured waveform demonstrating the net signal generated by the overlapping separate, high frequency iFU signals, whose two, dominating spectral peaks appear in the right-hand figure of B. The low-frequency waveform overlying on the high-frequency signals in the left-hand figure of B matches the observed beat frequency shown in the spectrum (inset of figure of B).

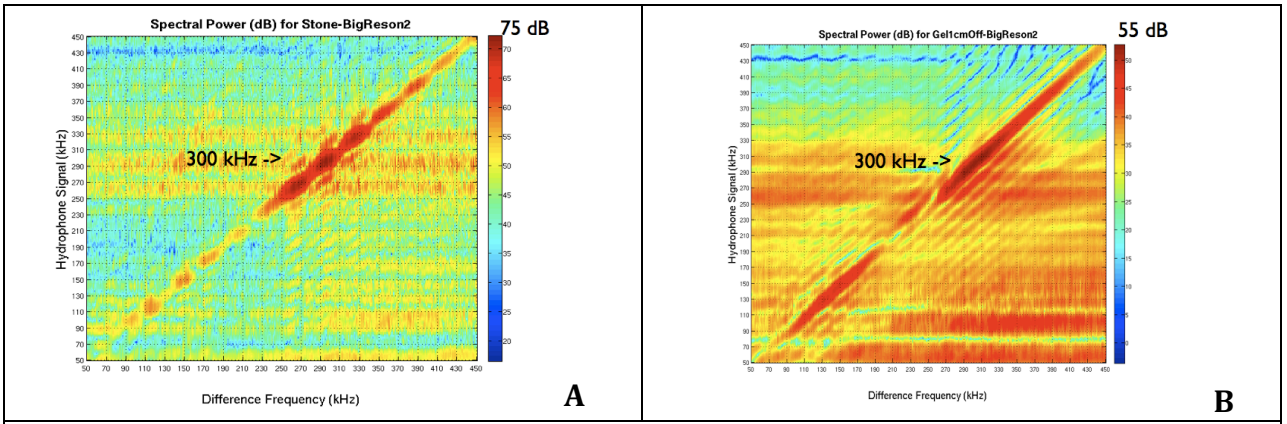
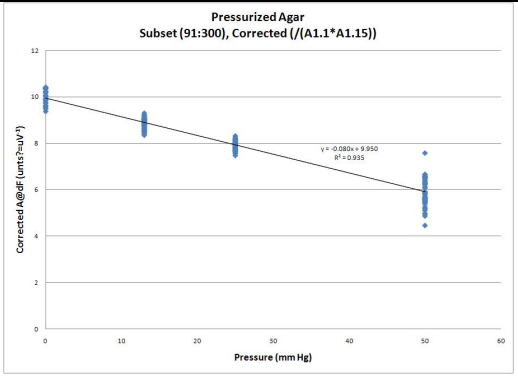


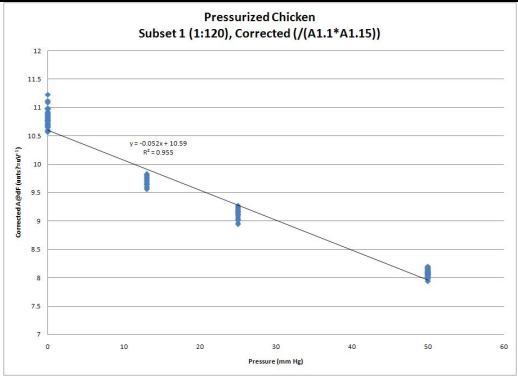
Figure 2. Each figure shows the amplitude of acoustic emissions generated by vibroacoustography applied to a tissue phantom that either **A** contains a calcium carbonate target or **B** does not. This is a positive control for the efficacy of our vibroacoustography methodology. Varying along the horizontal axis is the difference frequency between our two vibroacoustography carrier frequencies, here ranging from 50-450 kHz. Varying along the vertical axis is the frequency of the measured acoustic emissions, ranging from 50-450 kHz. Both experiments show comparable patterns of acoustic emissions, emblematic of vibrations induced in the entire *in vitro* system. Diagnostic here is the size of the acoustic emissions: much bigger when the calcium carbonate target lies at the focus of the iFU transducer (**A**) rather than away from the focus (**B**), by a multiplicative factor of over 100.

Milestone #1b. We will relate mathematical quantities describing that palpation to the pressure placed on model brain tissue in vitro.

We were able to assay how overpressure candidate tissue phantoms (agar; store-bought chicken; an acoustic absorber) changed the acoustic emissions generated within the phantom by iFU. Specifically, the acoustic emissions typically decreased as overpressure increased (see the example of Figure 3a,b,c). This is consistent with our guiding hypothesis, namely that the tissue phantom's ability to generate vibrations decreases as over-pressure increases. Critical here was normalization of the acoustic emissions data by the measured amplitude of the backscattered carrier ultrasound wave that brought the ultrasound-based momentum to the focal spot within the tissue phantoms.



A



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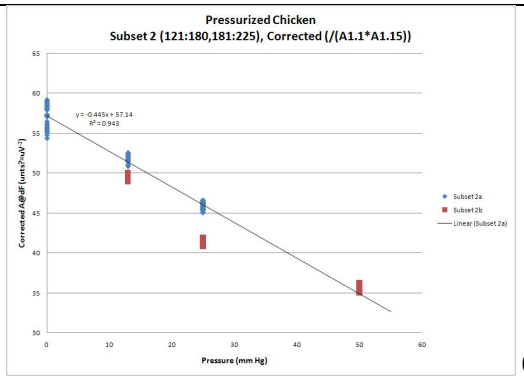


C

Figure 3. All figures show the amplitude of acoustic emissions (vertical axis) as a function of pressure applied to the target tissue (horizontal axis), with several experimental realizations per study. Examples **A-C** show our consistent results: as overpressure increased for a variety of tissue models the acoustic emissions decreased.

With regard to palpation studies *in vitro*, Figure 4a,b,c shows three time series of iFU-induced palpation at three values of overpressure, highlighting examples of what is shown statistically in Figure 4d: the amplitude of iFU-induced displacements decrease as overpressure increases. This is a large range in pressure 0-500 mmHg, ten times larger than is relevant in the clinic. These measurements are nonetheless useful because they point to a prediction of approximately 1-micron reduction in displacement per mmHg of overpressure, suggesting that we should be able to differentiate between 0 and 25 mmHg *in vivo,* which we tested below.

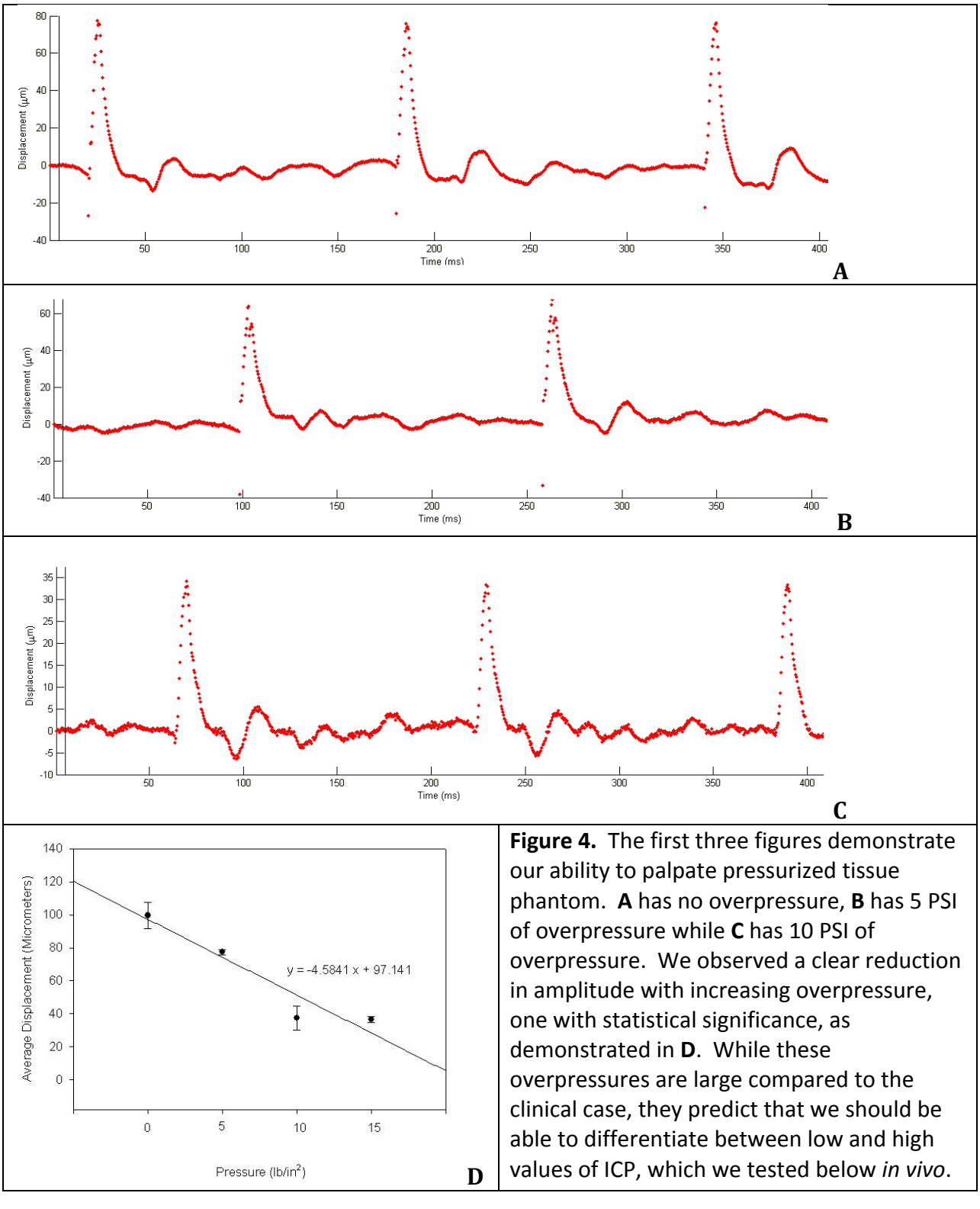


Figure 4. The first three figures demonstrate our ability to palpate pressurized tissue phantom. **A** has no overpressure, **B** has 5 PSI of overpressure while **C** has 10 PSI of overpressure. We observed a clear reduction in amplitude with increasing overpressure, one with statistical significance, as demonstrated in **D**. While these overpressures are large compared to the clinical case, they predict that we should be able to differentiate between low and high values of ICP, which we tested below *in vivo*.

Aim #2. We will demonstrate this methodology in a rodent model of elevated ICP.

Milestone #2a. We will establish an acute rodent model of elevated ICP.

We established our model using an implanted clinical invasive ICP sensor through one portal through the cranium while passing a tube filled with water through another. This proved to be a less reliable model than we had hoped. Specifically, the animals usually died when subjected to clinically meaningful ICP values. Moreover, it proved difficult to maintain tight control on the value of ICP due to leakage of fluid from the cranium. We intend to seek additional funding (NIH-R21 most likely) to take our same methodology and apply it to a different model of intracranial pressure, hoping for more consistent results.

Milestone #2b. We will relate mathematical quantities extracted from transcranial, iFU-mediated palpation of rodent brain to the pressure placed on that brain tissue.

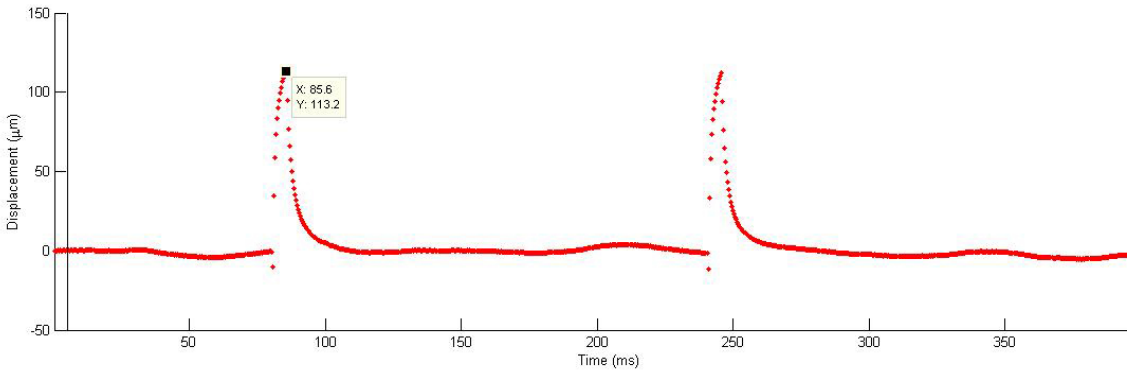
We applied ultrasound transcranially to rat brain in order to measure the focal displacement of rat brain versus intracranial pressure (the first kind of palpation discussed above). In particular, Figures 5a,b (overleaf) show a clear difference in amplitude of iFU-induced brain displacement. Figure 5c (overleaf) shows the associated statistics associated with measurements of the *peak* or *maximum* amplitude of brain-tissue displacement: there is a discernable trend towards reduced maximum displacement induced by iFU associated with increasing the overpressure within the rat's cranium, a difference within a factor of two of what our *in vitro* study predicted. However, the large variability in our measurements of brain displacement does not yet allow us to claim that we've observed a statistically significant correlation between overpressure and the amplitude of ultrasound-induced displacement. We attribute the variance in our measurements to the difficulties we found in reproducibly increasing the pressure within the cranium of our rats, as noted above.

We tried different kinds of analysis of the palpation data, but were unable to extract a statistically significant difference in low versus high values of ICP, despite observing encouraging trends. For example, we varied the number of data points we included in our analysis. We also interpolated the data of Figures 5a,b with single or double exponentials of the following forms.

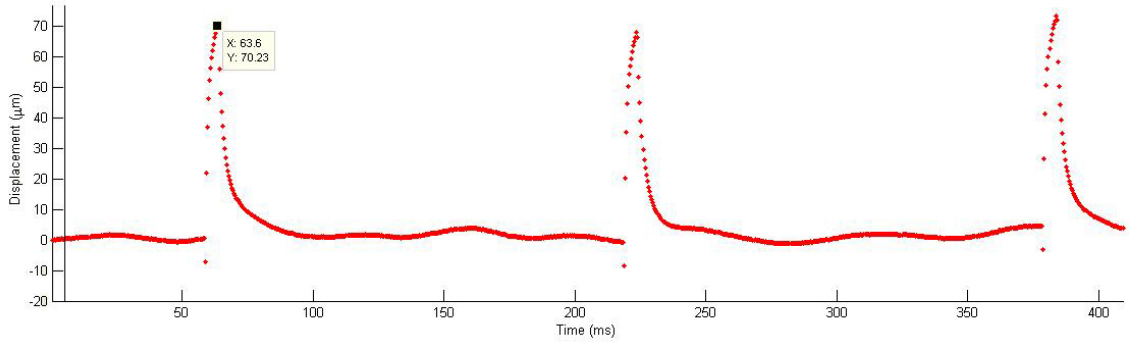
A*exp(-B*t) or

a_1 exp(-b_1*t) + a_2 exp(-b_2*t).

The double exponential represented the data better, because the wave forms of Figures 5a,b demonstrated a rapid fall off captured by the first exponential, while the second exponential captured the slower, longer and less step resolution of the displacement to its background value. Regrettably, regression on any of A, B, a_i, b_i did not produce a statistically significant predictor of ICP.



A



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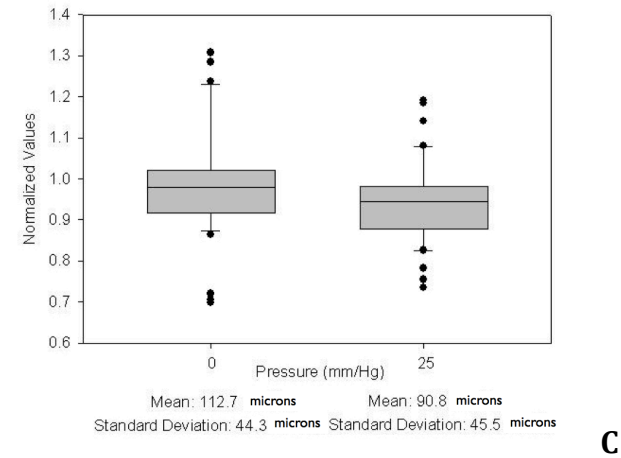

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Figure 5 shows the results of applying focused ultrasound to rat brain in a transcranial fashion for a clinically relevant range of intracranial pressure (here, 0-25 mmHg). **5A** shows representative displacements without overpressure (ICP = 0 mmHg) while **5B** shows representative displacements at ICP = 25 mmHg. There is a clear trend towards smaller amplitude of iFU induced brain displacement as overpressure increases (**5C**), though the difference does not yet approach statistical significance, because of the large variability in our results.

We next applied our vibroacoustography technique *in vivo*, discovering a statistically significant relationship between the amplitude of acoustic emissions induced by intense focused ultrasound and intracranial pressure (Figure 6). Specifically, we measured the acoustic emissions from rat brain transcranially, normalized each measurement by the detected amplitude of backscattered ultrasound at the carrier frequency, and averaged these results over a range of frequencies. We observed a decrease in those normalized acoustic emissions as a function of increasing intracranial pressure.

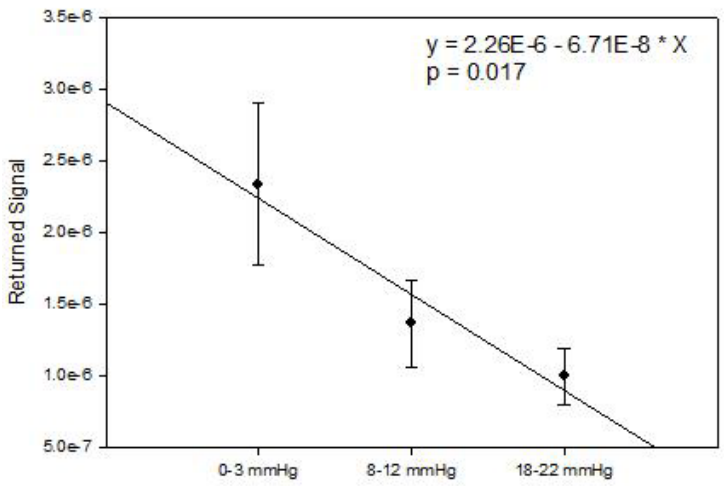

Figure 6. Amplitude of acoustic emissions generated by a vibroacoustography signal induced by intense focused ultrasound, normalized by the amplitude of the backscattered signal at the carrier frequency. Across a clinically meaningful range of increasing ICP, the acoustic emissions reduced by a multiplicative value of three. These results were robust, good for three different rats across nine different cycles of ICP.

Aim #3. We will demonstrate the safety of this methodology.

Milestone #3a. We will use the brains of rats in acute and survival studies to demonstrate the safety of iFU-mediated brain palpation by assaying for potential BBB disruption after application of iFU shown to palpate rat brain in a manner predictive of ICP.

Figure 7 shows a photo of a rat brain that received the same amount of ultrasound through a cranial window as caused observable displacements and vibrations, a conservative experiment for the safety of our ultrasound protocols for brain palpation. This is one of ten rat brains that received ultrasound to assay for the safety of our procedure. This rat brain did not show blood brain barrier disruption, which would have appeared as blue dots on the surface of the brain. Out of the 20 applications of iFU (2 per rat), only two showed blood-brain barrier disruption, these with an aspect, position suggesting they arose due to the surgical procedure. Since blood-brain barrier disruption constitutes the 'first' damage that appears after ultrasound application to brain, we feel confident that these protocols are safe.

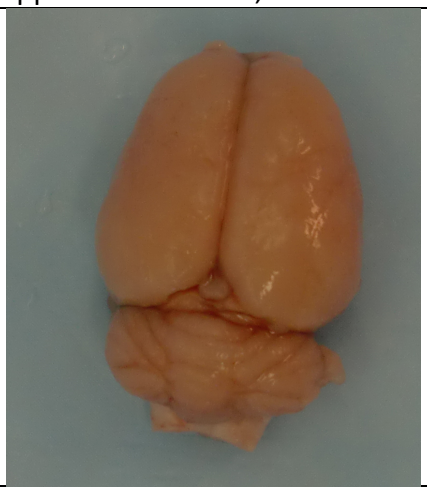

Figure 7. Dorsal view of a rat brain that had received the same amount of ultrasound (in each hemisphere) used to create observable palpations in pressurized tissue phantom (Figure 1) and *in vivo* (Figure 3, below). The first sign of damage we would expect to see is blood-brain barrier opening, which would appear as blue discoloration on the brain. While we saw that in other rats to which we applied more ultrasound, this rat and the other 9 rats to which we applied intense focused ultrasound did not show any iFU-induced blood-brain barrier disruption.

List of participating personnel.

Damon Cassisi. Mechanical engineer, engaged to refine experimental equipment.

Trevor Dickey. Biochemist and Engineer, engaged to run the animal experiments and analyze the data.

Dan Gross. Mechanical engineer, engaged to build and refine experimental equipment

and supporting software for running the experiments, as well as perform the experiments.

P. Ray Illian. Software engineer, engaged to refine the experimental equipment, primarily its software, as well as perform the experiments.

Pierre D. Mourad. Principal investigator, designing the experiments and helping to run the experiments.

REPORTABLE OUTCOMES

(1) tissue phantom subjected to a range of overpressure demonstrate that the amplitude of palpation induced by intense focused ultrasound (iFU) varies inversely with overpressure such that one can expect a change in displacement amplitude when applied *in vivo* by approximately 1 micron for every change in mmHg of overpressure.

(2) iFU for palpation induction applied to rat brain tissue *in vivo* subjected to a 25 mmHg change in overpressure demonstrates the predicted trend in amplitude of palpation versus overpressure without, however, achieving statistical significance. More sophisticated analysis did not change this outcome, arguing for an improved model of intracranial pressure in future work.

(3) tissue phantom subjected to a range of overpressure demonstrate that the amplitude of vibration induced by intense focused ultrasound (iFU) varies inversely with overpressure such that one can expect a reduction by 20% of the acoustic emissions generated by iFU applied *in vivo* over a clinically useful change in overpressure (by about 25-30 mmHg).

(4) iFU for vibration of rat brain transcranially showed a larger decrease in acoustic emissions as a function of intracranial pressure than anticipated *in vitro.* Those emissions reduced by 1/3 over a meaningful range of ICP.

(5) The iFU protocols used in this study do not disrupt the blood-brain barrier, demonstrating the safety of our procedure.

CONCLUSIONS

Ultrasound-induced vibration successfully correlated *in vivo* with intracranial pressure in a statistically significant fashion, using an ultrasound protocol that we observed to be safe as determined by our observation of damage-free brain using these protocols. Ultrasound-induced palpation of showed a correlation *in vivo* with intracranial pressure. Regrettably, the observations were not statistically significant – encouraging but by no means definitive results. As a result, using intense focused ultrasound as a means of detecting intracranial pressure in a transcranial fashion each shows promise in generating safe and measurable diagnostics for the non-invasive measurement of intracranial pressure. However, this must be demonstrated in a more reliable and reproducible animal model of intracranial pressure.

REFERENCES AND APPENDICES

We presented these results at the ISTU (International Society of Therapeutic Ultrasound) meeting in New York City in April 2011.